ORIGINAL RESEARCH

Evaluation of Fibrin Clot Interaction with Dental Implant after Different Surface Treatments: An *In Vitro* Study

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Abstract

Aim: The current study was carried out to assess the interaction between fibrin clots and dental implants following various surface treatments.

Materials and methods: In this investigation, 45 dental implants with dimensions of 16 mm in length and 5 mm in diameter were utilized. They were divided up into three groups, each consisting of fifteen samples. Group I: Control; Group II: Ultraviolet (UV) light treated; and group III: Sandblasted and acid-etching (SLA) treated. Healthy volunteers' venous blood samples were drawn into vacutainer tubes without the use of anticoagulants. The samples were centrifuged for 3 minutes at 2700 rpm in a table centrifuge. The entire implant was submerged in room-temperature liquid fibrinogen for 60 minutes. Then, scanning electronic microscopy (SEM) was used to examine each sample. The inter- and intragroup assessments were obtained using the Mann–Whitney *U* test and the Kruskal–Wallis test; *p*-values less than 0.05 were regarded as statistically significant.

Results: The maximum adhesion of fibrin clot was found in SLA treated group (2.42 ± 0.10) followed by the UV light-treated group (2.18 ± 0.08) and control group (1.20 ± 0.02). There was a statistically significant difference found between the three surface-treated groups ($p < 0.001$).

Conclusion: All surface-treatment methods exhibit adhesion between the implant surface and the fibrin clot. However, the highest adherence of fibrin clot was found in SLA treated group compared to the UV light-treated and control group.

Clinical significance: The physical and chemical characteristics of an implant's surface have a significant impact on the way blood clots organize. At the interface between the implant and the bone, blood clot production can initiate and facilitate the healing process.

Keywords: Dental implant, Fibrin clot, Surface treatment, Venous blood.

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Introduction

Dental implants are recognized as a safe treatment option for partially or fully edentulous patients, with high long-term survival rates. However, in other scenarios, such as poorly managed diabetes, tobacco use, bisphosphonate medication, and radiation therapy, osseointegration is less predictable. The establishment of a stable fibrin clot that permits the migration of various cells toward the bone-implant interface is a crucial and early stage in peri-implant hard tissue healing.[1]

Implant surfaces physiochemical properties like topography, wettability, and surface energy have been extensively researched and adjusted to promote osseointegration. A variety of biomimetic techniques have also been investigated for functionalizing implant surfaces through the use of growth factors, hydroxyapatite (HA), calcium phosphate, and bone morphogenetic proteins (BMPs) to encourage osteoinduction, osteoconduction, and osteogenesis.[2]

A blood clot that forms just around the implant occupies the peri-implant wound site initially. Connective tissue cells migrate through the remnants of the clot still attached to the implant surface, which has been modified by both ion and protein exchange. Blood contact with the implant surface sets off a series of events that start with the adsorption of serum or plasma proteins and proceed all the way to the recruitment and activation of cells.[3]

Ten minutes after blood contact, cellular components such as polymorphonuclear granulocytes appear. Blood contact activates platelets, which promotes healing, and creates a temporary

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biological matrix that allows osteogenic cells to move to the surface topography of the implant. Connective tissue cells that start wound contraction around the fifth day may be present in this temporary matrix.[4]

Sandblasting and acid-etching (SLA) is a commonly employed method in implant dentistry to enhance the rugosity of the material. Sandblasting the turned surface with large-grit (i.e., alumina) particles creates an SLA surface. Afterward, an acid is used to chemically modify the surface. To enhance the contact surface and osseointegration, the SLA surface topographically produces a roughness with big dips, sharp edges, and tiny micropits.[5] According to reports, UV irradiation improves the recruitment, adhesion, retention, proliferation, and general phenotype of osteogenic cells, hence decreasing surface hydrocarbon and increasing the hydrophilicity of the implant surface. There have been reports that the roughness of implant surface topography enhances bone cell proliferation and differentiation.[6] Additionally, a series of coordinated events, including protein adsorption, proliferation, and deposition of bone tissue, are probably affected by the different topography surfaces.[7] Therefore, a crucial step in the osseointegration process is the implant surface's ability to hold onto the fibrin net. Only a few studies are available on the fibrin clot interaction with dental implants. Hence, the present was conducted to assess fibrin clot interaction with dental implants after different surface treatments.

Materials and Methods

The current *in vitro* study was conducted in the Department of Periodontics and Oral Implantology at Kalinga Institute of Dental Sciences, Bhubaneswar, Odisha, India during the year 2023. Institutional approval was obtained. In this *in vitro* experiment, 45 dental implants with dimensions of 16 mm in length and 5 mm in diameter were utilized (ADIN Dental Implants, Israel).

The samples were randomly divided into the following three groups of 15 implants each, based on the surface treatment applied:

- **Group I: Control**—The surface was burnished and cleaned for a minute using a cotton pellet saturated with normal saline.
- **Group II: Ultraviolet-light treated**—15 samples were exposed to UV light for 15 minutes using a UV activation device. A single source with wavelengths of $\lambda = 360$ nm and $\lambda = 250$ nm was employed to provide a variety of wavelengths in the UV spectrum.
- **Group III: Sandblasted and acid-etching treated**—After polishing the surfaces with 3 µm diamond paste, the surfaces were roughened using 150 µm grain size Al_2O_3 and 6 bar of pressure. The surfaces were then acid-etched for 10 seconds with a 38–40% hydrofluoric acid (HF) solution. Following a 5-minute ultrasonic cleaning session with double-distilled water, all of the samples were let to air dry.

Preparation of Liquid Fibrinogen

Three healthy volunteers' venous blood samples (9 mL) were drawn into noncoated vacutainer tubes without the use of anticoagulants. The samples were centrifuged in a table centrifuge using the Andrade et al.[8] technique, which called for 3 minutes at 2700 rpm. The yellow liquid at the top of the white cap tubes (liquid fibrinogen) was aspirated with a sterile syringe, (avoiding red blood cells) and immediately transferred into Eppendorf tubes. Then, liquid fibrinogen was placed in three different containers. Each group's samples were immersed together (15 samples in each group with three different containers) a room temperature for a total of 60 minutes. Following this time, the implants were carefully taken out while maintaining the fibrin clot that was affixed to them. The samples were then preserved for 15 minutes in 1% formaldehyde phosphate-buffered saline. The samples were dehydrated by immersing them for 10 minutes in each of the serially diluted (25, 50, 75, 95, and 100%) ethanol solutions.

Evaluation of Fibrin Clot Using Scanning Electron Microscope

For 3 days, the samples were stored at room temperature in a desiccator container filled with dehydrated silica gel, labeled with metallic stubs. The samples were gold sputtered for 120 seconds to prepare them for scanning electronic microscopy (SEM), and an energy-dispersive spectrometer-equipped microscope was used for analysis. Backscattering microscopic pictures (40×) were chosen for the clot extension study. Following the calibration of the images and the adjustment of the delimited regions to the threshold, the measurement tool made it possible to calculate the percentage of clot area extension on the specimen surface. The area of the black points was automatically measured and summarized by the software. Each analysis was carried out twice by a blinded examiner.

Statistical Analysis

A computerized version 20.0 of the Statistical Package for the Social Sciences (SPSS) was used to carry out the statistical analysis. Descriptive statistics were used to analyze the data and determine whether they had a normal distribution. The inter- and intragroup assessments were obtained using the Mann–Whitney *U* test and the Kruskal–Wallis test; *p*-values less than 0.05 were regarded as statistically significant.

Results

Table 1 depicts the comparison of fibrin clot adhesion on dental implants after different surface treatments. The maximum adhesion of fibrin clot was found in the sandblasted and acid-etching (SLA) treated group (2.42 ± 0.10) followed by the UV light-treated group (2.18 ± 0.08) and control group (1.20 ± 0.02). There was a statistically

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Table 1: Comparison of fibrin clot adhesion on dental implant after different surface treatments

Surface-treatment groups	*Mean ± SD*	*95% CI for mean*		*Mean rank*	*p-value*
		Lower	*Upper*		
Group I: Control	1.20 ± 0.02	1.18	2.08	10.08	0.001
Group II: The UV light treated	2.18 ± 0.08	2.10	2.84	19.49	
Group III: The SLA treated	2.42 ± 0.10	2.32	2.78	21.26	

CI, confidence interval; SD, standard deviation; SLA, sandblasting and acid-etching; UV, ultraviolet

Table 2: Pairwise comparison between the different surface-treatment groups

Surface-treatment groups	*Mean difference*	*Significance*
Control vs UV light treated	−0.98	0.001
Control vs SLA treated	−1.22	0.001
UV light-treated vs SLA treated	−0.24	0.790

SLA, sandblasting and acid-etching; UV, ultraviolet

significant difference found between the three surface-treated groups ($p < 0.001$).

Table 2 shows the pairwise comparison between the different surface-treatment groups. There was a statistically significant difference found between Control vs UV light-treated and Control vs SLA treated groups with a mean difference of −0.98 and −1.22, respectively. However, there was a significant difference found between UV light-treated vs SLA treated groups with a mean difference of −0.24.

Discussion

The cells' adhesion to biomaterial surfaces is an important step in the biomaterial/tissue integration. This adhesive interaction anchors cells that activate several intracellular signaling pathways to direct cell viability, proliferation, and differentiation. As there is more surface area available for adhesion, the tissue response to rougher surfaces appears to be better than that to smoother ones.[9]

Dental implants are able to get attached to the alveolar bone through a process called osseointegration. The length of the implant and the surface topography of the implant are two significant elements that influence the osseointegration process. There are several methods to improve the dental implant's osseointegration, including altering or modifying the surface of the implant. A study by Sollazzo V et al.[10] claimed that an increase in surface roughness on the implant will result in a greater surface area, which will eventually boost both cell growth and proliferation. According to the study by Sehrawat M et al.,[11] any extra coating applied to the implant surface eventually increases its surface area and promotes successful implant osseointegration with the alveolar bone. In the present study, titanium dental implants with dimensions of 16 mm in length and 5 mm in diameter were utilized. Titanium has been determined to be the material of choice for dental implants due to its great biocompatibility, high strength, low modulus of elasticity, and high resistance to corrosion.[12]

For the cytoskeletal cell elements involved in spreading and motility, the altered surface topography exhibits a geometric feature that serves as a mechanical constraint. Dermal wound healing is a process that is accompanied by concurrent connective tissue cell migration and wound contraction. Fibrin is generated from the reaction of thrombin and fibrinogen released into the healing site. The peri-implant bone location may also experience this, which could lead to the temporary fibrin scaffold retracting from the implant surface. The healing and osseointegration processes appear to be improved in proportion to the extent of the clot that is kept on the implant surface.[13]

The scanning electron microscope is one of the most adaptable tools for characterizing chemical composition and examining and analyzing morphological microstructure. Through scanning electron microscopy (SEM), the surface properties following surface treatment and sample adhesion were assessed. It was demonstrated using SEM pictures that implants with various surface treatments have a rougher surface. This suggests that the surface area available for osseointegration has increased, resulting in a higher ratio of the implant surface to the bone, ultimately contributing to the implant's success.[14]

In the current investigation, the group that received sandblasting and acid-etching (SLA) treatment had the highest level of fibrin clot adherence when compared to the UV light-treated group and the control group. Similarly, Bhavanchand et al.[15] carried out an *in vitro* investigation to assess the titanium's hemocompatibility following surface treatment. After being exposed to titanium samples, the hemocompatibility test revealed a decrease in the platelet count, albeit it was still within ISO guidelines. It was determined that varied surface modifications had no effect on the hemocompatibility of medical-grade titanium. There was a noticeable increase in surface roughness, which boosted the implant's adherence to fibrin clots.

These results are similar to those of Gahlert M et al.,[16] who demonstrated that acid etching and airborne particles both raised surface roughness. The surface topography could change together with the physics and surface chemistry. Surface roughness can be similar across machined implant surfaces and surface modifiers such as sandblasting or acid etching.

Hyeongil Kim et al.[17] conducted another investigation on implants treated with SLA and these results were in accordance with the present study. Large grits were used for SLA of the implants' surface. To promote osseointegration, it expanded the implants' surface. An SEM and profilometer were used to scan the titanium's topographic surface for further analysis. Upon SLA-treated implants, tiny, homogeneous micropits with a diameter of 1–2 μm were seen.

The process of blasting is crashing minute particles, like alumina, ceramic, titanium oxide, and CaP particles, together to form a porous layer that covers the implant surface. In this study, 150 μm-sized particles of Al_2O_3 were employed. Similarly, Wennerberg et al.[18] stated that grit blasting with different sizes of Al_2O_3 particles altered the topography of commercially pure titanium, which similarly enhanced bone formation at the implant.

A limitation of the present study could be the observational nature of the data and the small number of implant surfaces examined in the current investigation. Future studies should take into account other implants with unique nanosurfaces, such as laser-microtextured surface (LMS), which have demonstrated favorable clinical outcomes in preserving the alveolar bone, achieving a high standard of tissue sealing, and averting peri-implantitis. To assess additional variables that can affect biomimetic functionalization and investigate the practical implications of these findings for the osseointegration process and success rate of dental implants inserted using this method, more research is required.

Conclusion

Within the limitation, the present study concluded that all surface-treatment methods exhibit adhesion between the implant surface and the fibrin clot. However, the highest adherence of fibrin clot was found in the SLA treated group compared to the UV light-treated and control group.

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